

Virus occurrence in private and public wells in a fractured dolostone aquifer in Canada

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Abstract Groundwater samples from 22 wells completed in a regional fractured dolostone aquifer in the Guelph region of southern Ontario, Canada, were collected over an 8-month period and analyzed for viruses and *Campylobacter jejuni*. Only 8% of the 118 samples exhibited viruses at extremely low concentrations, but of the 22 wells sampled, 10 (45%) were positive for human enteric viruses (polyomavirus, adenovirus A, and GII norovirus) including 5 of the 8 public supply wells (62.5%) and 5 of the 11 private wells (45%). Each virus-positive well had only one virus occurrence with six sampling events during the 8-month sampling campaign and only one virus type was detected in each well. The probability of virus detection was positively associated with well open-interval length. Virus concentration (in the wells that were virus-positive) was negatively associated with well depth and open-interval length and positively associated with overburden thickness (i.e., the thickness of unconsolidated materials overlying bedrock facies) and the amount of precipitation 8–14 and 15–21 days prior to the sampling date. The ephemeral nature of the virus detections and the low detection rate on a per sample basis were consistent with previous studies. The percentage of virus-positive wells, however, was much higher than previous studies, but consistent with the fact that the hydrogeologic conditions of fractured bedrock aquifers create

wide capture zones and short groundwater travel times to wells making them more vulnerable to contamination occurrence but at very low concentrations.

Keywords Fractured rock · Viruses · Water supply · Canada · Health

Introduction

Of the 818 drinking-water outbreaks recorded by the Centers for Disease Control and Prevention in the United States between 1971 and 2008, approximately a third (248) were linked with the consumption of untreated groundwater, and of these, 45 (26.2%) occurred in vulnerable hydrogeologic settings such as karst (Wallender et al. 2014). In Canada, the tragedy in Walkerton, Ontario, where seven people died and more than 2,300 people became ill when *Escherichia coli* (*E. coli*) and *Campylobacter jejuni* contaminated the municipal water supply (O'Connor 2002) drew the attention of Canada to the first issues concerning pathogens in groundwater, particularly in bedrock aquifers. The case study of a norovirus outbreak in Wisconsin (Borchardt et al. 2011) highlights the uniqueness of virus contamination of groundwater in a fractured bedrock aquifer as the virus exhibited rapid transport from a large septic system to a restaurant's supply well in less than 1 week leading to over 200 individuals becoming infected with norovirus. In these cases and many others, the pathogenic outbreaks resulted from sewage-derived or manure-derived contamination of drinking-water supplies. When combining the results from 15 large-scale studies investigating the occurrence of sewage-derived pathogens and fecal indicators in public water systems, the United States Environmental Protection Agency (US EPA) projected that approximately 23.6% of all public supply wells across the United States will

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be contaminated with human enteric viruses at some point in time (US EPA 2006).

Lab-scale studies conducted since the late 1970s have determined the various influences on virus inactivation and mobility in natural groundwater systems. To date, it has been shown that virus longevity in groundwater is significantly increased by decreased microbial antagonism (Hurst 1988; Davies et al. 2006), low groundwater temperatures (Yates et al. 1985; Charles et al. 2009), and increased soil moisture content (Yeager and O'Brien 1979). As summarized by Zhang et al. (2012), virus mobility in the subsurface has been shown to occur as colloidal transport which makes the virus particles in fractured media less prone to typical sources of attenuation in porous media such as diffusion, adsorption to grain surfaces, or size and charge exclusion effects, and therefore promotes virus particles to exhibit transport speeds on the order of calculated average linear groundwater velocities assuming fracture porosities for rock. As effective porosities on the order of 10^{-3} to 10^{-5} in fractured rock promote large groundwater velocities (Freeze and Cherry 1979) and colloids have shown transverse dispersion coefficients up to 4 orders of magnitude greater than those of the solutes (Weisbrod et al. 2013), virus transport in fractured bedrock aquifers can be both rapid and widely distributed in a matter of days. While sediments overlying fractured bedrock aquifers are expected to offer some attenuation of viruses before they enter the aquifer, the predominant sources of enteric viruses are leaking sewers and septic systems that are commonly buried several meters below ground surface and in close proximity to the top of rock or down in trenches dug into the rock to maintain slopes for gravity flow. With shallow water tables and cool temperatures promoting virus longevity up to and potentially longer than 1 year (Charles et al. 2009) and groundwater velocities on the order of 10 m/day typical of fractured dolostone aquifers (Belan 2010), active viruses can potentially be transported several kilometers from a single source while maintaining their ability to infect a host. Despite the potential for rapid and widespread distribution of viruses in groundwater, to date, the number of studies investigating the presence of actual viruses in fractured bedrock aquifers is extremely limited (Table 1).

In the first studies investigating the transport of viruses through fractured bedrock, Borchardt et al. (2007) and Bradbury et al. (2013) demonstrated the vulnerability of the Mount Simon sandstone aquifer to virus contamination through the regionally extensive Eau Claire shale aquitard. In the 2007 paper, Borchardt et al. conducted monthly sampling of three public water supply wells in Madison WI to reveal the presence of human enteric viruses in two wells that were cased through the Eau Claire aquitard and open to the underlying confined sandstone aquifer. Similarly, Bradbury et al. (2013) sampled six wells all of which exhibited virus detections. The studies ultimately revealed that, while failed

well casings could act as cross-connections from the upper unconfined aquifer to the lower confined aquifer, simultaneous detection of viruses in wells several kilometers apart would require several well casings to fail at the same time. As this scenario was unlikely, it was confirmed that preferential flow paths from the upper Wonewoc aquifer through the Eau Claire aquitard were likely present in the form of naturally occurring fractures and acted as the means for virus transport to the lower confined aquifer, therefore highlighting the fact that not even confined bedrock aquifers were impervious to virus contamination. While these studies confirmed the vulnerability of fractured bedrock aquifers to virus contamination, the number of wells and therefore type of wells investigated were relatively low.

In two studies conducted by Davis and Witt (2000) and Femmer (2000), over 200 Missouri municipal supply wells completed in the carbonate and karstic carbonate aquifers of the Ozark Plateaus were sampled for human enteric viruses. While the large array of wells exhibited varying constructions, ages, and surrounding land use and therefore allowed for the evaluation of the various factors contributing to virus contamination, each of the wells was only sampled 1 or 2 times. In Bradbury et al.'s 2013 study, the importance of temporal sampling is highlighted as even wells highly prone to virus contamination did not always exhibit viruses throughout over a year of monthly sampling events (the well with the highest number of positive virus detections had detections during only 61% of sampling events). Other studies sampling a wide array of wells such as Lindsey et al. (2002) and Johnson et al. (2011) also completed a limited number of sampling events at each of their wells and so detection rates are not representative of the actual vulnerability of the wells or aquifers. Studies including temporal sampling such as those conducted by Lieberman et al. (2002), Borchardt et al. (2003), and Locas et al. (2007), therefore, provide better insight with regard to the true vulnerability of a well completed in a fractured bedrock aquifer. In the study by Lieberman et al. (2002), temporal sampling of 13 wells completed in karstic bedrock was conducted over an entire year and found that 43% of the wells exhibited culturable viruses. In the study by Locas et al. (2007), the only known field-scale studies of viruses in Canadian groundwater, both of the bedrock wells that were sampled also exhibited viruses. While both of these studies produced temporal data, Lieberman et al. only sampled wells that had previously recorded contamination with microbial water quality indicators such as *E. coli* and Locas et al. (2007) sampled only a limited number of bedrock wells. In comparison, the investigation by Borchardt et al. (2003) conducted temporal sampling of a wide array of wells in a large variety of settings. Although Borchardt et al. (2003) temporally sampled 30 wells completed in bedrock and revealed that 10% of them were susceptible to virus contamination, all the wells were private wells and so no estimate for larger municipal supply wells was made.

Table 1 Summary of investigations of virus occurrence in fractured bedrock aquifers

Reference	Study Location	Approximate area of study site (km ²)	Type of bedrock	Number of wells		Sample frequency	Total No. of samples	Virus detection rate per well (%)			
				AW	BRW			By PCR		By cell culture	
								AW	BRW	AW	BRW
Davis and Witt 2000	Ozark Plateaus	122,000	Mix of CA and KCA	109	109	Annually for 2 years	218	12	12	0.9	0.9
Femmer 2000	Ozark Plateaus	122,000	Mix of CA and KCA	109	109	Once	106	0	0	NA	NA
Lindsey et al. 2002	Pennsylvania	119,283	25 CA; 25 CY	59	50	Once	50	NA	NA	13.6	8 (CA); 4 (CY)
Lieberman et al. 2002	USA	9,826,675	Mixed	30	13 (7 Karst)	Monthly for 1 year	360	NA	NA	23	Karst: 43
Borchardt et al. 2003	Wisconsin	169,639	Mixed	50	30	Quarterly for 1 year	194	8	10	0	0
Borchardt et al. 2007	Madison WI	243.5	SS	3	3	10 Monthly samples	30	66	66	33	33
Locas et al. 2007	Quebec	1,542,056	UB, SS	12	2	Monthly for 1 year	113	25	0	33	100
(Bradbury et al. 2013)	Madison WI	243.5	SS	6	6	Monthly from 9/2007 to 4/2009	26	100	100	100	100
Johnson et al. (2011)	East Tennessee	~64,800	KCA	4 (and 4 springs)	4	1–2 times, timing unknown	6	0 ^a	0	75	75

AW all wells, BRW bedrock wells, CA carbonate, CY crystalline, KCA karstic carbonate, SS sandstone, UB undefined bedrock, NA indicates that PCR analysis not conducted

^a Results from PCR analysis suggested by authors to be false negatives due to sample collection and concentration techniques

Despite the fact that each of the studies summarized in Table 1 sampled wells completed in fractured rock, a single, large-scale investigation focusing on the temporal susceptibility of various types of wells completed solely in fractured bedrock aquifers and the numerous factors contributing to their vulnerability has not been reported in the literature.

The goal of the current study is to assess the vulnerability of the regional fractured bedrock aquifer supplying drinking water to the residents and communities in southern Wellington County, Ontario to contamination with enteric viruses and determine the factors that promote this susceptibility. Over an 8-month period, 22 private, municipal supply, and monitoring wells situated in the Silurian dolostone aquifer were sampled monthly for enteric viruses including five human viruses (enterovirus, hepatitis A, norovirus genogroups I and II, polyomavirus, and adenovirus), bovine polyomavirus, and influenza A virus. To provide the study with several types of scenarios that are typical to both urban and rural settings, each of the wells chosen to be sampled exhibited different characteristics with regards to well completion depths and pumping rates, with variable sediment types and thicknesses, and proximity to different potential sources such as agriculture, surface-water bodies, sanitary sewers, and septic systems. As this study area is covered with glacially derived sediments with varying degrees of thickness and permeability in addition to the cool groundwater temperatures and rapid groundwater velocities on the order of 1–10 m/day (Belan 2010), it is expected that all well types completed in this fractured Silurian dolostone aquifer will be vulnerable to contamination with animal and human sewage-derived enteric viruses.

Materials and methods

Description of study area

The array of 22 sampling wells (Table 2) is located approximately 100 km west of Toronto, Ontario, Canada in various urban and rural communities of southern Wellington County (Fig. 1). The area surrounding the City of Guelph (population: 130,000) is dominated by agriculture and small towns. Of the 2,511 farms located in Wellington County in 2011, there were 363 dairy cattle and milk production farms, 423 beef cattle ranching and farming farms, 120 hog and pig farms, and 166 poultry and egg farms, making Wellington County one of the largest agricultural hubs in Ontario. These farms host a total of 142,197 cattle and calves, 236,144 pigs, 5,706,394 hens and chickens, and 248,811 turkeys. To the south of Guelph are two agricultural and residential areas, Arkell and Aberfoyle (combined population: 7,000) and to the northeast is Eden Mills (population: 350) built along the banks of the Eramosa River. Northwest of Guelph are Fergus (population 19,000) and Elora (population: 4,000), the two largest communities within

Centre Wellington Township. As Guelph, Fergus, and Elora are larger communities, they have both public water distribution systems and sanitary sewers serving the majority of their populations with septic systems and private wells on the outskirts of the villages and rural areas. Despite the availability of municipal drinking water works in the older sections of town, many residents of Fergus and Elora still rely on private wells and septic systems even within highly populated centres of the communities. The populations of Arkell, Aberfoyle, and Eden Mills are entirely reliant upon private wells and septic systems.

Although all of the communities chosen for this investigation are entirely encompassed by the 6,800 km² of the Grand River watershed, the belt of Silurian dolostone that subcrops beneath the glacial sediments along the Niagara Escarpment and extends from Lake Huron/Georgian Bay to Niagara Falls, Ontario serves as a regionally important aquifer for nearly 800,000 people in southern Ontario including over 200,000 residents of Wellington County, and therefore, groundwater is the primary source of drinking water in the study area. All of the wells chosen for this investigation were completed in the Lower Silurian sequences as described by Brunton (2009) and consist of the Guelph, Eramosa, Goat Island, Gasport, Irondequoit, Rockway and Merriton formations and regionally bounded by the underlying Cabot Head shale Formation. Both the Guelph and Gasport formations act as the predominant fractured bedrock aquifers in the region. In the study area, the Guelph Formation is characterized by crinoidal grainstones and wackestones, is generally 15–22 m thick, and exhibits a sharp basal contact with the shaley facies and crinoidal grainstones of the underlying Eramosa and Goat Island formations, respectively. Although the Eramosa and Goat Island are water-bearing formations, their relatively lower transmissivities cause them to be considered regional aquitards (Golder Associates 2006). When vertical fractures and preferential pathways in the form of cross-connecting boreholes are not present, the Eramosa and Goat Island formations can limit the transport of some surface-derived contaminants present in the unconfined Guelph aquifer to the underlying Gasport aquifer. The thickness and presence of the Eramosa and Goat Island formations, however, are highly variable in the study area and these formations are sometimes entirely absent (Belan 2010). The 25–70 m of the blue-grey crinoidal grainstones of the Gasport Formation can therefore be in direct contact with the overlying Guelph Formation, which in turn is overlain by various types of glacial deposits that potentially offer a source of attenuation of surface contaminants before they enter the rapid flow regime of the underlying fractured bedrock aquifers. The presence and thickness of both of the Port Stanley and Wentworth Tills is highly variable in the study area throughout southern Wellington County (Fig. 3b,c) and studies investigating the vulnerability of the underlying fractured bedrock aquifers have established

that the varying thicknesses and degrees of permeability of these sediments result in the aquifers being highly susceptible to surface contamination (Lake Erie Region Source Protection Committee 2012).

Selection of wells for sampling

The 22 wells sampled during this investigation (Table 2; Fig. 2) consisted of existing private (PW), municipal supply (MSW), and monitoring (MW) wells completed in the local fractured dolostone aquifers within a 20-km radius of the City of Guelph. Each of the wells was chosen in order to represent the range of well construction and hydrological conditions that might affect the vulnerability of typical bedrock wells to virus and bacterial contamination and the variety of well constructions and pumping volumes common to the study area. Surficial geology data were obtained from Ontario Ministry of Northern Development and Mines (2013). Final selections were based on seven criteria: (1) well type, i.e., private, municipal supply, or monitoring well; (2) well completion, including total depth, length of open interval, and bedrock aquifer segment; (3) the well's proximity to potential sources such as sanitary sewers, septic systems, animal agriculture operations, or surface water bodies; (4) the spatial distribution across the study area and flow system; (5) surrounding land use, i.e., urban versus rural; (6) overburden thickness (i.e., the thickness of unconsolidated materials overlying bedrock facies); and (7) accessibility/permission.

Using these criteria alongside the Ontario Ministry of the Environment's Well Record Database, 17 wells in the towns of Fergus, Elora, Arkell, Eden Mills, and Aberfoyle were chosen to be sampled. In addition to the aforementioned criteria and the Well Record Database, sewer invert elevations in Guelph were plotted relative to the top of bedrock (Fig. 3) and used to select five wells in areas where sewers were constructed into or near the top of bedrock. In the end, a total of 22 wells including 11 private wells, 8 municipal supply wells, and 3 monitoring wells were chosen to be sampled. The characteristics of each well are summarized in Table 2 and so only a brief summary of the wells will be presented here.

In Centre Wellington, six municipal supply wells were sampled, including three wells in Elora and three wells in Fergus. Each well was cased through the overburden sediments into the upper meter or so of the Guelph Formation. The wells were therefore open from the top of the Guelph Formation to varying depths within the Gasport Formation. In contrast to the wells sampled in Centre Wellington, those sampled within the City of Guelph did not consist of any actual municipal supply wells but two large capacity water supply wells operated by the University of Guelph and three monitoring wells. The three 2-inch (5 cm) diameter monitoring wells with varying completion depths are located along the Speed River at a site of known industry-derived

Table 2 Details of the eight large supply wells, three monitoring wells, and 11 private wells that were sampled in this project

Location	Well ID Total depth (m) Completion year	Overburden type Thickness (m)	Open interval (m)	Formation	Well diameter (in)	Use	Land use/potential sources	Approximate distance from potential sources (m)	Unique well characteristics improving diversity of well array
Elora	MSW1 129.8 1948	Gravel 9.45	9.4–130	GU, GA	8	M	R, SS, SL	30 m from sewer	In close proximity to both urban sewers and septic systems
	MSW2 121.9 1991	Diamicton 13.1	15–121.9	GU, GA	10	M	A, I, SS, M	<30 m from agriculture; 100 m from sewers	Surrounded by agriculture but also in close proximity to automotive manufacturer with large and diverse staff contributing to sewer effluent
	MSW3 128 Unknown	Diamicton 25	25–128	GU, GA	10	M	A, SS, M	<10 m from agriculture; 500 m from septic systems	Exceptionally deep well surrounded by agriculture
	MSW4 110 1965	Gravel 4.88	5.5–110	GU, E, GI, GA	16	M	B, R, SS, SL, SW	25 m from Grand River; <50 m from sewer	In close proximity to both urban sewers and septic systems and adjacent to the Grand River
Fergus	MSW5 129.5 1972	Sand 78.9	80.5–129.5	E, GA	12–12–10 telescopic	M	I, R, A, SS, SL	25 m from sewer; 300 m from agriculture, 100 m from industry	Located within industrial area approximately 400 m east of residential area served primarily by sewers
	MSW6 124.4 1975	Gravel 15.2	34.1–124.4	GU, E, GI, GA	12–10 telescopic	M	R, A, P, SS, SL	300 m from park septic; 50 m from sewer; 50 m from agriculture	In close proximity to residential area served by sewers and septic systems. Located across the street from a high school of approximately 1,500 students and 300 m from a community-sized septic system serving a local sportsplex and fairground
	MW1 8.4 1999	Sand 1.8	4–8.361	GU	2	MW	R, P, B, SS, SW	<10 m from sewer; ~10 m from Speed River	Cased through overburden and open to Guelph Formation only. In close proximity to main trunk of Guelph sewer system along Speed River and site with historic VOC contamination
	MW2 11.3 1991	Sand 1.8	8–11.287	E	2				Cased through overburden and Guelph Formation. Open only to Eramosa Formation. In close proximity to main trunk of Guelph sewer system along Speed River and site with historic VOC contamination
U. of Guelph	MW3 25.4 2000	Sand 1.8	18.5–25.4	GA	2				Cased through overburden, Guelph, and Eramosa Formations. Open only to Gasport Formation. In close proximity to main trunk of Guelph sewer system along Speed River and site with historic VOC contamination
	MSW7 58.8 1958	Diamicton 12.8	12.8–58.8	GU, E, GI, GA	10	R	U, R, SS	<10 m from sewer	Supply well with pumping rate of ~280 L min ⁻¹ on University of Guelph campus with dense and diverse population contributing to sewers
	MSW8 87.8 1953	Gravel 20.4	14.3–87.8	GU, E, GA	10	R	R, B, SS	<10 m from sewer	Supply well with pumping rate of ~280 L min ⁻¹ located in restaurant parking lot ~ from centre of University of Guelph campus. Completed less than 10 m from sewers constructed less than 8 m above the top of bedrock and 12 m above the top of the well's open interval
	PW1 29 2006	Gravel 27.1	27.4	GU	6.25	D	A, R, SL	~30 m from septic systems; ~500 m from agricultural	Shallow private well cased through 27.1 m of gravel overburden and open to Guelph Formation. Surrounded by agriculture and residential septic systems
Aberfoyle	PW2 54.9 1991	Gravel 23.2	24.1–54.9	GU, E, GA	5	D	A, R, SL	~15 m from septic systems	Deep private well cased through 23.2 m of gravel overburden and open to Guelph, Eramosa, and

Table 2 (continued)

Location	Well ID Total depth (m) Completion year	Overburden type Thickness (m)	Open interval (m)	Formation	Well diameter (in)	Use	Land use/potential sources	Approximate distance from potential sources (m)	Unique well characteristics improving diversity of well array
Arkell	PW3 24.4 1992	Gravel 21.3	21.9–24.4	GU	6	D	A, R, SL	~15 m from septic systems	Gasport formations. Surrounded by agriculture and residential septic systems Shallow private well cased through 21.3 m of gravel overburden and open to Guelph Formation. Surrounded by agriculture and small community relying on individual septic systems
	PW4 59.4 1986	Gravel 15.8	16.5–59.4	GU, E, GA	5	D	A, R, SL	~15 m from septic systems	Deep private well cased through 15.8 m of gravel overburden and open to Guelph, Eramosa, and Gasport formations. Surrounded by agriculture and residential septic systems
	PW5 20.4 1981	Gravel 15.2	15.2–20.4	GU	6	D	A, R, SL	~15 m from septic systems	Shallow private well cased through 15.2 m of gravel overburden and open to the Guelph Formation. Surrounded by agriculture and small community relying on individual septic systems
	PW6 20.4 1977	Gravel 13.4	15.85–20.45	E	5	D	A, R, SL	~15 m from septic systems	Shallow private well cased through 13.4 m of gravel overburden and open to only the Eramosa Formation. Surrounded by agriculture and small community relying on individual septic systems
	PW7 74.7 1988	Diamicton 11.3	12.19–74.676	GU, E, GI, GA, CH	6	D	A, SL	<200 m from septic systems	Deep private well cased through 11.3 m of diamicton overburden and open from the Guelph to the Cabot Head Formation. Located on a golf course and surrounded primarily by agriculture and some septic systems
	PW8 32 2006	Gravel 7.3	7.62–32.0	GU, E	6	D	A, SL	~15 m from septic systems	Moderately deep private well cased through 7.3 m of gravel overburden and open to the Guelph and Eramosa Formations. Surrounded primarily by agriculture and some septic systems
	PW9 67.1 2007	Gravel 14.9	15.8–67.1	GU, E, GI, GA	6	D	A, SL	~15 m from septic systems	Deep private well cased through 14.9 m gravel overburden and open to the Guelph, Eramosa, Goat Island, and Gasport formations. Surrounded by agriculture and small community relying on individual septic systems
Eden Mills	PW10 15.8 2003	None 0.1	12–15.8	GA	6	D	A, R, SL, SW	~15 m from septic systems	Shallow private well located in small community relying on septic systems along the Eramosa River where overburden is absent and fractured bedrock is exposed. Well is cased through 12 m of bedrock and open to the Gasport Formation
	PW11 31.1 1985	None 0.3	15.2–31.1	GA	5	D	A, R, SL, SW	~15 m from septic systems	Moderately deep private well located in small community relying on septic systems along the Eramosa River where overburden is absent and fractured bedrock is exposed. Well is cased through 15.2 m of bedrock and open to the Gasport Formation

Formation abbreviations: *GU* Guelph, *E* Eramosa, *GI* Goat Island, *GA* Gasport, *CH* Cabot HeadWater use abbreviations: *M* municipal, *MW* monitoring well, *R* research, *D* domesticSurrounding land use abbreviations: *R* residential, *A* agricultural, *I* industrial, *P* public parks, *B* business, *U* university campusPotential sources abbreviations: *SS* sanitary sewer, *SL* septic leaching, *SW* surface water, *M* manure application

Note: 1 in = 2.54 cm

contamination with various volatile organic compounds and within 10 m of the main trunk line of Guelph's municipal sanitary sewer system that was constructed below the local water table and up to 4 m below the top of rock. The two University of Guelph water supply wells located within the centre of the City of Guelph are both 10-inch (25 cm) diameter wells and are pumped at an approximate rate of 280 L min⁻¹. For the purpose of this study, these wells are considered comparable to municipal supply wells and are labeled as such.

Each of the 11 private wells was located in a small residential or agricultural community outside the City of Guelph and had a unique well completion. In the small Puslinch communities of Arkell and Aberfoyle, four and five private wells of varying completion depths were sampled, respectively. The remaining wells added to the study were two private wells of different total depths and completion years located along the Eramosa River in Eden Mills.

Sampling methods

Sampling began in the second week of June 2012 and extended until January 2013. Each of the wells was sampled once for general chemistry and several times for enteric pathogens within the 8-month sampling period. Most wells were sampled monthly for 6 months, while other wells were sampled less frequently due to limited accessibility. Temporal sampling for viruses is required as previous studies have shown virus concentrations in groundwater to be extremely variable (Bradbury et al. 2013). During virus sampling events in July and November 2012, samples for bacterial fecal indicators, *E. coli* and total coliforms, were also collected.

For the current study, all virus samples were collected by following a standard operating procedure (SOP) developed in accordance with the procedures outlined in Millen et al. (2012). Prior to sampling, all equipment set to come in contact with raw well water was soaked in a 0.52% NaClO solution for 30 min prior to being rinsed with 0.05 M sodium thiosulfate for 5 min. All hoses and tubes were then flushed with three volumes of DI water and covered with parafilm for transport to the sampling site. Samples from municipal supply wells were taken directly from raw water taps within each of the well houses, whereas private wells were sampled from external faucets bypassing water treatment such as filtration units or water softening systems. Monitoring wells were sampled using a Grundfos Redi-Flo2 electrical submersible pump. For all three well types, sampling hoses were connected to a flow-through cell with a YSI 556 multiprobe system to monitor field parameters including pH, temperature, and conductivity during purging. Once these parameters were constant, glass wool filters (Lambertini et al. 2008) were attached to well taps and faucets by sterile half-inch tubing. At one site known to have VOC contamination, filtered water was collected into 55-gal drums and treated at an onsite treatment

centre. To maximize virus adsorption to glass wool well water pH levels ≥ 7.5 were adjusted to between 6.5 and 7.0 by injecting 0.5 M HCl before the glass wool filter using a Masterflex precision peristaltic pump. Four of the 22 study wells required pH adjustment during sampling. Mean sample volume was 877 L \pm 210 l (± 1 SD), range 483–9,16 L, $n = 118$.

Laboratory methods

Glass wool filters were packed on ice and shipped overnight to the USDA Agriculture Research Service in Marshfield, WI for analysis using the procedures outlined by Borchardt et al. (2012). Immediately upon arrival glass wool filters were eluted using 3% beef extract containing 0.05 M glycine (pH 9.5) in the direction opposite of sample flow to maximize collection of bacterial and viral targets (Abd-Elmaksoud et al. 2014). Eluate pH was adjusted to 7.0–7.5 using 1 M HCl and flocculated with polyethylene glycol 8000 (8% wt/vol) and NaCl (final concentration, 0.2 M). This mixture was stirred for 1 h, incubated overnight at 4 °C, and centrifuged at 4200 $\times g$ for 45 min. The pellet was resuspended in 2 ml of sterile 0.15 M N₂HPO₄ and the final concentrated sample volume stored at -80 °C.

Samples were analyzed by qPCR on a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) for six human viruses (adenovirus, enterovirus, norovirus genogroups I and II, hepatitis A virus, and polyomavirus) bovine polyomavirus, influenza A virus, and *C. jejuni* following the reverse transcription and PCR procedures described in Borchardt et al. 2012. Primers (IDT, Coralville, IA) and hydrolysis probes (TIB Molbio, Berlin, Germany) are reported in Table 3. PCR inhibition was measured for every sample following the methods of Gibson et al. (2012). Among 118 samples, six were PCR-inhibited; inhibition was mitigated by 1:5 dilution with nuclease-free water. No-template controls were included for every batch of analysis steps: nucleic acid extraction, reverse transcription, and PCR. All no-template controls were negative—i.e., no cycle quantification (Cq) value. Performance parameters of the standard curves are reported in Table 4. Standards consisted of g-blocks (targets > 125 base pairs) and ultramers (targets \leq 125 base pairs) both supplied by IDT. To facilitate identifying amplicon contamination from the standards, the sequence between primer and probe was scrambled while maintaining the wildtype base composition. One standard of each target with a Cq value near 30 was used as the positive control for every PCR analysis batch.

Glass wool filter performance was evaluated twice with samples collected from sites MW1 and MW3. Poliovirus Sabin type 3 was seeded into the samples and recovery measured following the methods described in Lambertini et al. (2008). Percent recovery for sites MW1 and MW3 were 53 and 39%, respectively, both which fall within the recovery

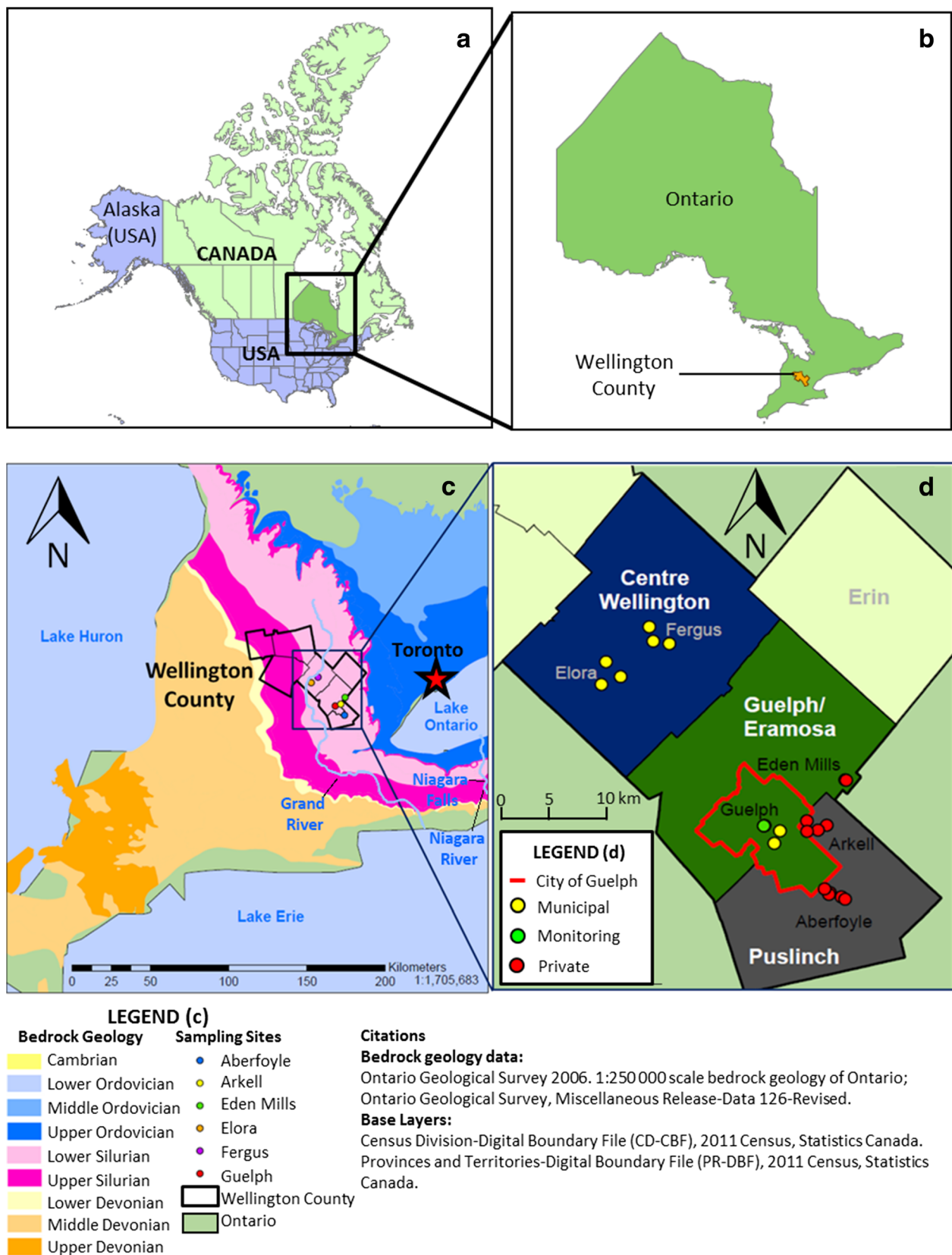


Fig. 1 **a–b** Sampling locations in the City of *Guelph*, Ontario, Canada, and several surrounding communities within *Wellington County*. **c** Bedrock geology of *Wellington County*, which is dominated by a Silurian dolostone belt extending from *Lake Huron* to the *Niagara River*. All sampling wells are constructed into the Lower Silurian carbonate formations including the *Guelph*, *Eramosa*, *Goat Island*, and *Gasport* formations. **d** Locations of sampled wells

range for enteroviruses concentrated by glass wool filtration (Lambertini et al. 2008).

Collection of groundwater samples for *E. coli* and total coliforms closely followed the guidelines set forth by US EPA Method 1604 (2002). As groundwater concentrations of *E. coli* and total coliforms were expected to be low, sample volumes were increased from the suggested 100 to 1,000 ml. Groundwater samples for bacterial analyses were collected in triplicate into autoclaved 1-L HDPE bottles and transported on ice to the University of Guelph.

Samples for *E. coli* and total coliform analyses were analyzed within 24 h of their collection using vacuum filtration following EPA Method 1604 (2002). Each groundwater sample was filtered onto pre-sterilized 47-mm diameter, grid-marked, cellulose ester membranes and placed on Oxoid CM 1038 Differential Coliform (DC) agar. After 24 h of incubation at 35 °C, the plates were removed and checked for bacterial growth. Pink colonies were counted as total coliforms and blue colonies were counted as *E. coli*. The plates were incubated for an additional 24 h followed by another round of colony counting. Blank samples were run between each filtration for quality control.

Data analysis

The association between well characteristics and virus levels was initially evaluated by Fisher's exact test on a 4×2 table where the rows were categories of overburden type (gravel, diamicton, sand, none) and the columns represented the dichotomous response (virus detected versus virus not detected). The same analytic procedure was then undertaken separately for each of three continuous-scaled characteristic variables (total depth, overburden thickness, open interval length) to examine their relationship with virus levels. First, the response was treated as dichotomous and a parametric log-binomial regression model was fit to estimate the probability of virus detection across the observed range of values for the well characteristic variable (McNutt et al. 2003). To determine the appropriate representation (linear, quadratic, etc.) of the well characteristic in the log-binomial model, a preliminary step was undertaken where a graphic derived from a nonparametric generalized additive model (GAM) was examined (Hastie and Tibshirani 1990). In the second analysis for each continuous-scaled well characteristic, the data were restricted to wells where virus was detected and robust regression techniques were used to evaluate the association between the characteristic and continuous-scaled virus concentration levels (Huber 1973).

Precipitation data were acquired from the Guelph Turfgrass Institute and the Elora Research Station, University of Guelph. For each date on which sampling took place, precipitation levels were tabulated at lags of 0–7, 8–14 and 15–21 days prior to the sampling date; analyses were carried out

separately for the three lag categories. The unit of analysis was a sampling date, and the same analytic approach as described above (GAM, log-binomial regression, robust regression) was used to examine the relationship between precipitation level and virus detection/concentration, except that a different robust regression technique was employed to accommodate the specific characteristics of the data (Yohai 1987). All analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Of the 22 wells sampled in southern Wellington County, 10 were positive for human enteric viruses, ranging in concentrations from 0.1 to 16 genomic copies per L (Table 5). Five of these wells were private wells, while the other five were municipal supply wells. Five out of 11 (45%) private wells that were sampled exhibited virus detections, while 5 out of 8 (62%) municipal supply wells were virus-positive. The three viruses that were detected included human polyomavirus, adenovirus A, and GII Norovirus (Fig. 4). None of the positive wells tested positive on more than one occasion nor did they exhibit more than one type of virus.

The association between type of overburden at the well locations and virus detection was marginally significant ($p = 0.09$), where more viruses were detected in gravel and diamicton. Overburden thickness and well depth were not significantly associated with the probability of detecting viruses. The estimated relationship in both cases indicated somewhat of a threshold effect (Fig. 5); in the log-binomial model for over-burden thickness one outlier was excluded, a well with overburden thickness of 78.9 m and no viruses detected. In the wells that were virus-positive, overburden thickness was positively associated with virus concentration (slope = 0.0965, $p = 0.029$) and a significant negative association was present for well depth (slope = -0.0199 , $p = 0.005$; Fig. 5). Wells with longer open intervals were more likely to be virus-positive ($p = 0.044$), and virus concentration was negatively associated with open interval length in wells where virus was detected (slope = -0.0177 , $p = 0.028$; Fig. 5).

Precipitation amount was not significantly associated with the probability of virus detection for any of the three lag periods evaluated (i.e., cumulative precipitation within 0–7, 8–14 and 15–21 days prior to the sampling date). The estimated relationships for the lag periods assumed different shapes but generally reflected positive associations (Fig. 6). On sampling dates with ≥ 1 virus-positive well ($n = 7$), virus concentration was positively associated with cumulative precipitation amounts in the periods 8–14 days (slope = 0.1690, $p = 0.033$) and 15–21 days before sampling (slope = 0.2127, $p = 0.012$; Fig. 6). The

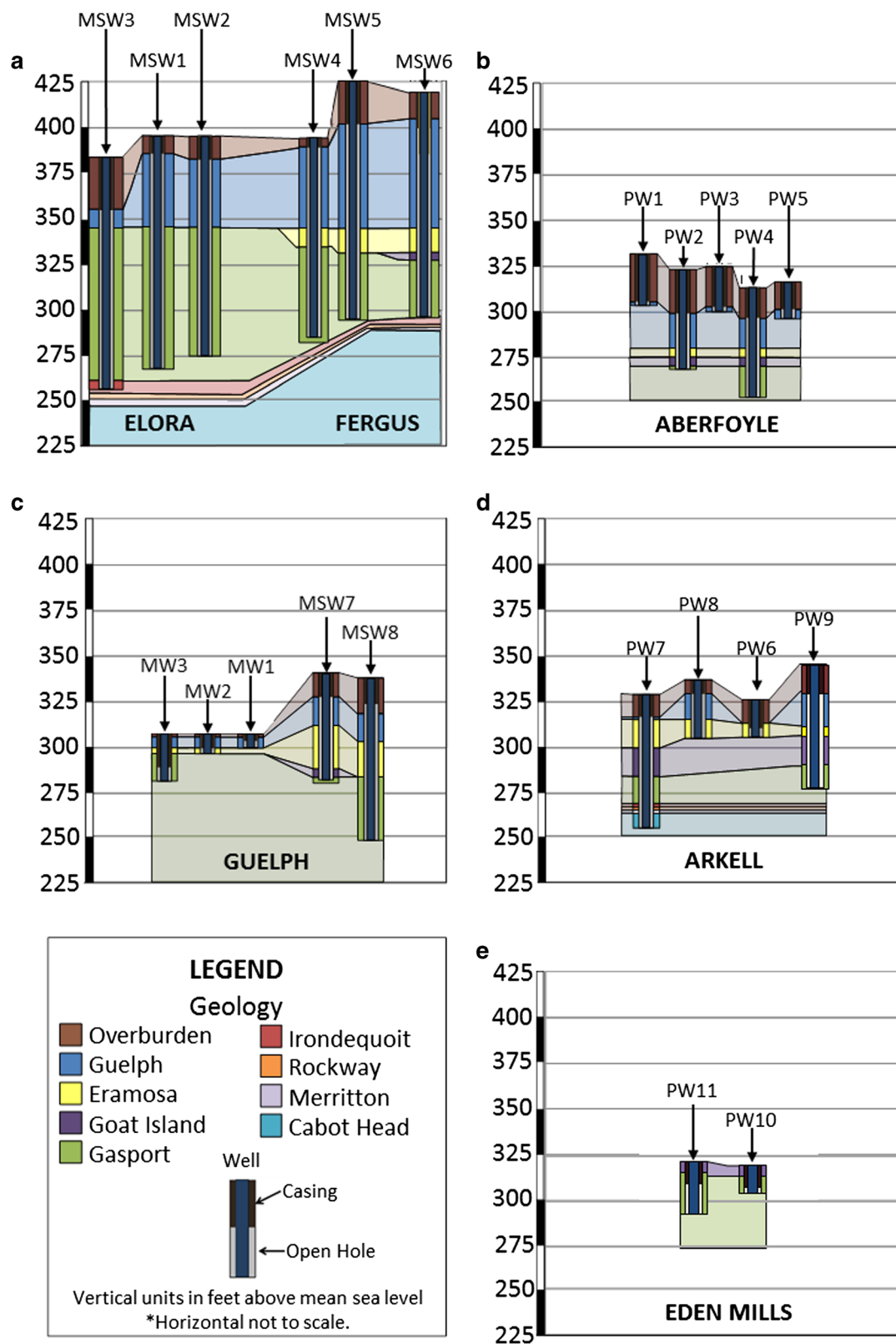


Fig. 2 Cross-section of sampling wells located across southern Wellington County illustrating well completions and bedrock geology. Although several of the wells are open in multiple formations, some are cased into a single hydrogeologic unit. *MW1*, *PW1*, *PW3*, and *PW5* are solely open to the Guelph Formation, with casing being set into the top of

rock. Wells *MW2* and *PW6* are open to the Eramosa Formation, while *MW3*, *PW11*, and *PW10* are open only to the Gasport Formation. **a** Centre Wellington sampling wells. **b** Aberfoyle sampling wells. **c** City of Guelph sampling wells. **d** Arkell sampling wells. **e** Eden Mills sampling wells

relationship between virus concentration and the amount of precipitation 0–7 days prior to sampling was positive but not significant (slope = 0.0916, $p = 0.457$; Fig. 6).

E. coli was detected in six out of the 22 wells (27%) sampled in this investigation including 1 municipal supply well, 2 monitoring wells and 3 private wells. All six wells with detectable *E. coli* would be above the Canadian Drinking Water Guideline of No Detections 100 m L⁻¹ for non-disinfected groundwater. While two wells were positive for *E. coli* during both sampling events, the other four positive wells only had detectable *E. coli* in one of the sampling events. The presence of *E. coli* was not always an indicator of virus detection. Only one well that was positive for human polyomavirus also contained detectable levels *E. coli*; additionally, the two wells exhibiting the highest concentrations of *E. coli* did not exhibit any viruses.

Total coliforms were detected in 11 out of 22 wells (50%) including 3 municipal supply wells, 2 monitoring wells, and 6 private wells. Total coliform concentrations were significantly higher than *E. coli* with 2 private wells exhibiting too many colony-forming units to count. Of the 11 wells to exhibit positive detections of total coliforms, five were also positive for human viruses, including human polyomavirus (3) and adenovirus A (2).

Discussion

Vulnerability of the dolostone aquifer

Previous assessments of the Grand River watershed suggested that the dolostone aquifers of the Guelph area exhibit a medium-to-high vulnerability to contaminants introduced at the ground surface due to the combination of permeable overburden with spatially variable thicknesses and the highly fractured nature of the bedrock aquifers (Lake Erie Region Source Protection Committee 2012). The detection of viruses in these aquifers is, perhaps, therefore not surprising. This 2012 assessment, however, is not specific to pathogen contaminants and has inconsistencies where vulnerability is discussed for this fractured rock aquifer where fracture porosities have been estimated to be in the 10⁻⁴ range (Belan 2010; Munn 2012) and yet the report used effective porosities of 10⁻¹ (1%) for estimating the 2-year well-head-protection areas, a reasonable estimate of virus longevity in Guelph area groundwater. In comparison to other studies, the detection of viruses in 10 out of the 22 wells sampled in this investigation is the highest detection rate of human enteric viruses in groundwater samples. On a per sample basis, however, only 10 of 118 virus samples exhibited detectable concentrations of viruses. While the ephemeral nature of virus contamination has been well established in the literature, the low per sample detection frequency was unexpected for the rapid flow regime

and highly vulnerable setting of the fractured bedrock aquifers of Guelph, Ontario; furthermore, when viruses were detected, the concentrations were consistently very low (1–16 genomic copies L⁻¹) in comparison to typical values found in wastewater (10⁴–10⁶ genomic copies L⁻¹; Bradbury et al. 2013). This suggests strong dilution due potentially to large well capture zones creating large amounts of mixing and/or weak inputs from sources during the study period.

Vulnerability of private wells

In the current investigation, five out of the 11, or 45% of private wells sampled, exhibited human enteric viruses including human polyomavirus, adenovirus A, and GII norovirus. These findings suggest that private wells completed in the fractured Silurian dolostone aquifer of the greater Guelph area are several times more vulnerable to virus contamination than private wells in Wisconsin where the only other study that had investigated virus contamination of private wells completed in fractured bedrock aquifers has been conducted (Table 6; Borchardt et al. 2003). The difference in well contamination rates between the two studies is likely not attributed to well depth, casing depth, sample volume, groundwater pH, or groundwater temperature as these parameters were similar between the two studies. Well age was much younger in the study by Borchardt et al. (2003), but well age in the current study was not associated with detection rates. Two key differences between the studies are the types of fractured bedrock aquifers investigated and the sampling frequency. In the current study, dolostone aquifers were sampled five or six times over eight consecutive months. Borchardt et al. (2003) also examined igneous and sandstone aquifers, and sampled each private well once per quarter over a calendar year. Virus contamination in wells is highly transient (Bradbury et al. 2013); therefore, the increased frequency of sampling on a monthly interval in this study may have contributed to a higher detection rate.

Vulnerability of municipal supply wells

The municipal supply wells of the study area also had detectable levels of viruses. Relying on 15 studies of viruses in groundwater, the US EPA (2006) estimated that a randomly selected municipal supply well in the United States had a 23.6% probability of virus contamination during its operational lifetime. In the current study, five out of eight (62%) large capacity supply wells exhibited viruses during the 8-month sampling period. Note, the EPA's estimate relied on cell culture techniques for virus detections, whereas the current study relied on qPCR, which tends to yield higher virus contamination rates compared to cell culture because it can detect inactivated viruses and a greater number of virus types.

Other studies have investigated the occurrence of viruses in municipal supply wells completed in fractured bedrock

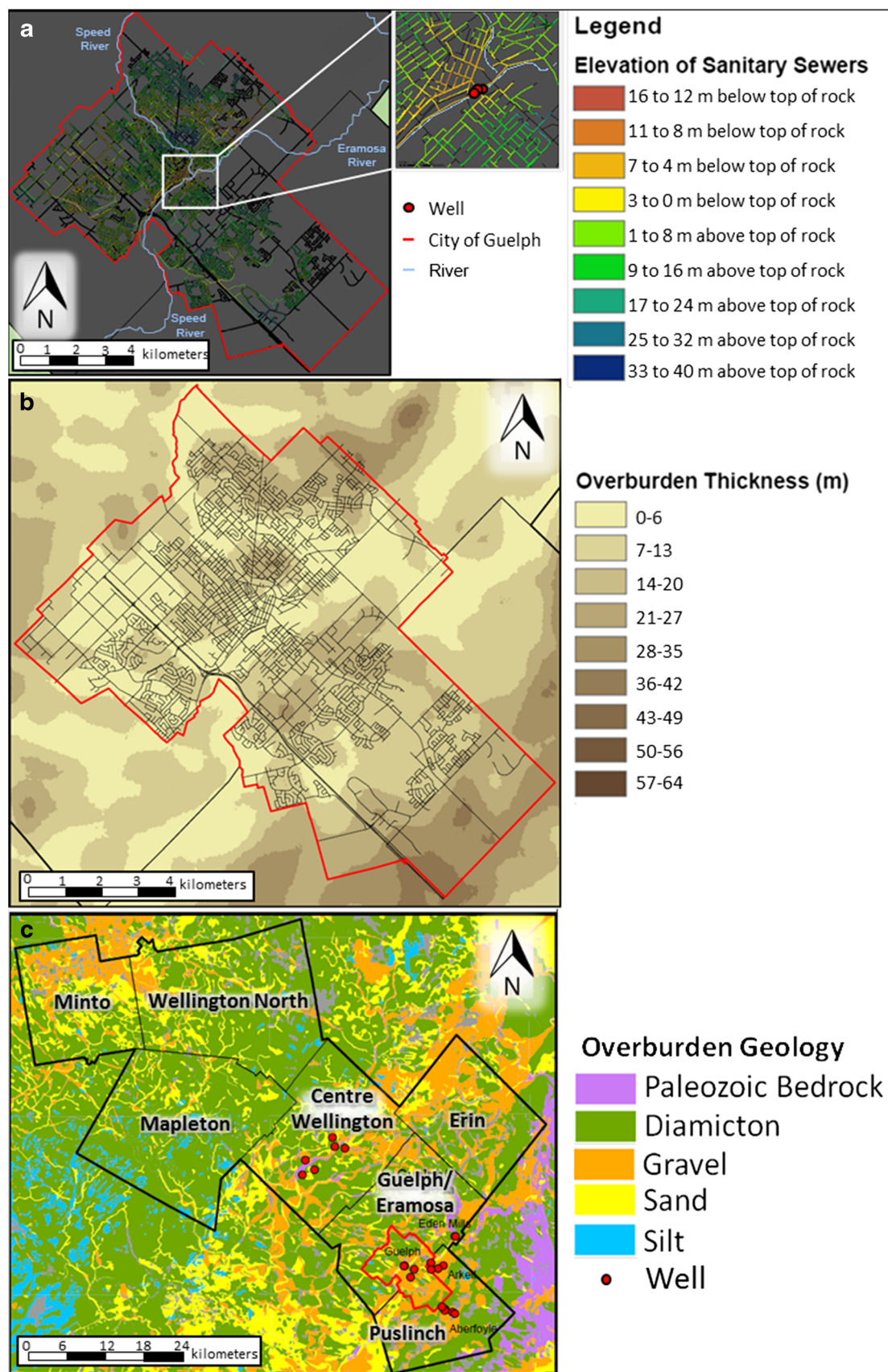


Fig. 3 Surface conditions influencing well selection. **a** Elevations of sanitary sewer inverts in relation to the top of bedrock. *Inset* illustrates sewer inverts below the top of bedrock at the location of monitoring wells (red dots) along the Speed River, **b** Overburden thickness, **c** Overburden type

aquifers (summarized in Table 1). While the studies in the Ozark Plateaus (Davis and Witt 2000; Femmer 2000) and Pennsylvania (Lindsey et al. 2002) sampled a wide array of wells, the virus detection rates are suspected of being low due to insufficient sampling frequencies of each well. In contrast, studies by Lieberman et al. (2002), Borchardt et al. (2007),

Locas et al. (2007), and Bradbury et al. (2013) each collected monthly samples in order to account for the temporal variability and short term occurrence of viruses in groundwater. The well contamination rate for viruses in the current study, 62%, is on the high end of the range reported for the previous nine North American studies.

Table 3 qPCR primers and probes used in this study. Primer final concentrations (nM) in master mix are noted *in parentheses*. Probe final concentrations are all 100 nM. Fluorophore and quencher for hydrolysis probes are 6-FAM and BBQ

Microbe	Primer or probe	Primer or probe sequence (5'–3')	Target gene	Amplicon size (base pairs)	Reference
Human adenovirus C, D, F	AdV-263-fwd	GGAGGCCTCGGAGT ACCTGA (500 nM)	Hexon gene (major capsid protein)	263	Kuo et al. 2009
	AdV-263-rev	CGCCGCGGATGTCA AAGTA (500 nM)			
	AdV-263-probe	CACCGATACGTACT TCAGCCTGGGT			
Human adenovirus A	AdV A - fwd	GGAGGCCTCGGAGT ACCTGA (500 nM)	Hexon gene (major capsid protein)	290	Kuo et al. 2009
	AdV A - rev	GCTTAAACTGGGA CCRCG (500 nM)			
	AdV A - probe	TGCGTTTGTGCCC GTGGAT			
Human adenovirus B	AdV B - fwd	GGAGGCCTCGGAGT ACCTGA (500 nM)	Hexon gene (major capsid protein)	255	Kuo et al. 2009
	AdV B - rev	ATGT CAAAGAAHGTGCTGG- CC (500 nM)			
	AdV B - probe	ACCCACGATGTGAC CACCGA			
Human enterovirus	EV-fwd	CCTCCGGCCCTGAATG (300 nM)	5' UTR	196	De Leon et al. 1990; Monpocho et al. 2000 (probe)
	EV-rev	ACCGGATGGCCAAT CCAA (900 nM)			
	EV-probe	CGGAACCGACTACT TTGGGTGTCCGT			
Hepatitis A virus	HAV-fwd	CTCCAGAATCATCTCCAA (700 nM)	VP1 and VP3 interface (viral capsid proteins)	192	Schwab et al. 1995
	HAV-rev	CAGCACATCAGAAA GGTGAG (700 nM)			
	HAV-probe	AATGTTTATCTTC AGCAATTAATCTGGA			
Norovirus genogroup I	JJV1-F	GCCATGTCCGTTGGATG (250 nM)	ORF1-ORF2 junction	95	Jothikumar et al. 2005
	JJV1-R	TCCTTAGACGCCAT CATCAT (250 nM)			
	JJV1-P	TGTGGACAGGAGAT CGCAATCTC			
Norovirus genogroup II	COG2F	CARGARBCNATGTTYAG- RTGGATGAG (400 nM)	ORF1-ORF2 junction	97	Kageyama et al. 2003
	COG2R	TCGACGCCATCTTC ATTCACA (400 nM)			
	Ring2-TP	TGGGAGGGCGATCG CAATCT			
Human polyomavirus JCV and BKV	SM2-fwd	AGTCTTTAGGGTCT TCTACCTTT (500 nM)	T antigen region (tumor antigen)	176 BKV 173 JCV	McQuaig et al. 2009
	P6-rev	GGTGCCAACTATG GAACAG (500 nM)			
Bovine polyomavirus	KGJ3-probe	TCATCACTGGCAAACAT	VP1 (major capsid viral protein)	79	Wong and Xagorarakis 2011
	BPyV-fwd	TGGCTTTCTGACTC AGCCAAA (500 nM)			
	BPyV-rev	TCTCTCTGAGAG TCACAGACATG (500 nM)			
Influenza A virus	BPyV-probe	ACCAACAGCAATTT AGAGGCCTTCCCAG	Matrix gene	99	Spackman et al. 2002
	M + 25 Fwd	AGATGAGTCTTCTA ACCGAGGTCG (500 nM)			
	M-124 Rev	TGCAAAAACATCTT CAAGTCTCTG (500 nM)			
	M + 64 (probe)	TCAGGCCCTCAA .AGCCGA			

Table 4 Quality assurance parameters for the qPCR standard curves

Target	Efficiency	Error ^a	Highest Cq standard measured
Human adenovirus groups C,D,F	1.896	0.118	40
Human adenovirus group A	1.914	0.0134	39.4
Human adenovirus group B	1.825	0.149	40
Human enterovirus	1.912	0.0407	40
Norovirus genogroup I	1.913	0.0225	40
Norovirus genogroup II	1.961	0.0279	40
Hepatitis A virus	1.959	0.0343	39.6
Human polyomavirus	1.927	0.0228	40
Bovine polyomavirus	1.888	0.00997	40
Influenza A virus	2.009	0.0204	40
<i>C. jejuni</i>	1.956	0.00686	40

^a According to the LightCycler 480 manufacturer an acceptable error value must be < 0.2

Groundwater flow systems influences on virus detection

The fractured Silurian dolostone aquifer in this study area (southern Wellington County, Ontario) are more susceptible to virus contamination and mobilization than aquifers composed of porous media. The individual grains of porous media aquifers provide greater surface area and thus more sites available for virus adsorption and attenuation compared to fractured bedrock aquifers. Additionally, groundwater velocities within porous media are much slower and may result in virus inactivation before transport has been completed from the source to the vulnerable well.

Belan (2010) and Munn (2012) investigated the hydraulic properties of the Guelph and Gasport formations that are the main water-supply aquifer in southern Wellington County, Ontario using various combinations of hydraulic tests and core logging techniques. Using FLUTE transmissivity profiling (Keller et al. 2014) in conjunction with ALS temperature logging (Pehme et al. 2010), packer tests (Quinn et al. 2011), and a 13-day pumping test, Belan (2010) estimated that the

transmissivity of the bulk aquifer (i.e., the Guelph and Gasport formations combined) ranged from 2.5×10^{-5} to 6×10^{-4} m² s⁻¹ and that fracture apertures ranged from 9 to over 430 µm with a mean of 69 µm. At the same study site, Munn (2012) completed a more detailed 3-D characterization of the fracture network using a suite of high-resolution, depth-discrete data collection methods including core logging, borehole geophysics (acoustic televiewer), and hydraulic testing (FLUTE transmissivity profiling) in 2 inclined coreholes and several nearby vertical coreholes. This yielded estimates of transmissivity between 2.0×10^{-4} to 4.2×10^{-4} m² s⁻¹ and fracture apertures between 15 and 407 µm with a slightly larger geometric mean between 104 and 159 µm in comparison to the estimates made by Belan. While Belan estimated an effective fracture porosity of the bulk aquifer on the order of 5×10^{-4} , Munn suggested the mean effective fracture porosity of the Guelph formation is approximately 1×10^{-3} and between 3×10^{-4} and 5×10^{-4} for the Gasport Formation. Such low effective fracture porosities are what lead to rapid groundwater velocities and transport times associated with

Table 5 Summary of the 10 virus detections in the southern Wellington County study area. GC L⁻¹ Genomic copies per litre

Date of detection	Well ID	Well type	Location	Virus	Concentration (GC L ⁻¹)
7/15/2012	PW4	Private	Aberfoyle	Human polyomavirus	1.52
7/24/2012	MSW4	Municipal supply	Fergus	Adenovirus A	0.09
8/12/2012	PW1	Private	Aberfoyle	GII norovirus	3.34
8/12/2012	PW3	Private	Aberfoyle	GII norovirus	15.16
8/12/2012	PW2	Private	Aberfoyle	Human polyomavirus	1.16
8/14/2012	MSW7	University supply	Guelph	Adenovirus A	1.23
8/20/2012	MSW6	Municipal supply	Fergus	Adenovirus A	0.54
9/11/2012	PW7	Private	Arkell	Human polyomavirus	1.99
11/7/2012	MSW1	Municipal supply	Elora	Human polyomavirus	1.01
11/7/2012	MSW2	Municipal supply	Elora	GII norovirus	15.63

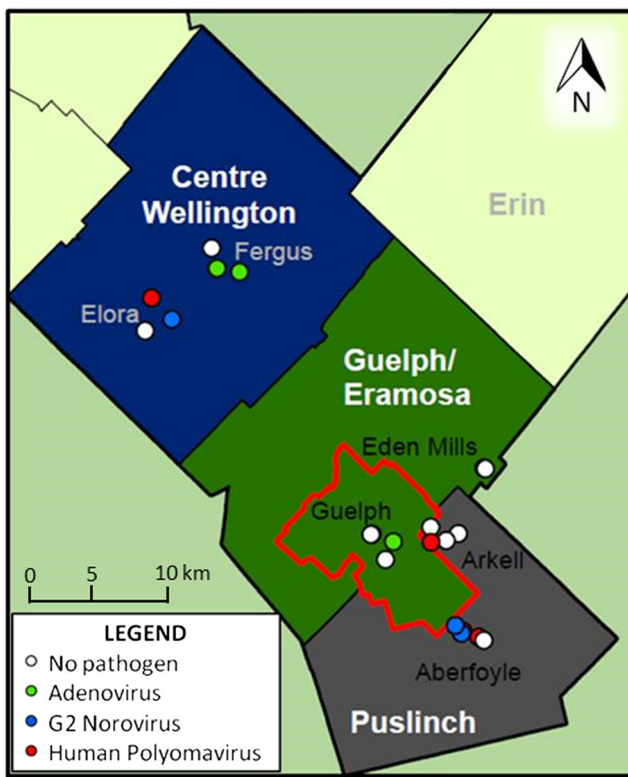


Fig. 4 Virus occurrence throughout southern Wellington County in 10 out of 22 wells. Each positive well only exhibited the presence of viruses once throughout the entire sampling schedule

fractured bedrock aquifers. As estimated by Belan (2010), the hydraulic conductivity and groundwater velocities of the fractured Silurian dolostone aquifer of southern Wellington County range from 6×10^{-6} m/s to 9×10^{-6} m s⁻¹ and from 0.002 to 28 m day⁻¹ under natural gradients, respectively. With fracture apertures 10^3 – 10^4 times larger than virus particles, groundwater hydraulic conductivities and velocities promoting transport up to 10 km in a single year, limited virus adsorption due to the limited surface area of fracture apertures and cool groundwater temperatures that favor virus survival, the intrinsic vulnerability of wells completed in fractured bedrock aquifers is much higher than wells completed in porous media aquifers regardless of well construction and surrounding land use.

The type of overburden material will likely influence virus attenuation and transport to the underlying aquifer. In this investigation, virus detections were greatest in wells surrounded by sediments composed of gravel or diamict, consistent with the rapid groundwater velocities characteristic of these sediments. Although a well may be located in an area of tight diamict and therefore relatively low permeability, the high mud content makes it susceptible to having fractures, providing preferential pathways for rapid groundwater flow and transport of viruses and bacteria from sources (Cherry et al. 2006), therefore increasing the well's vulnerability to contamination.

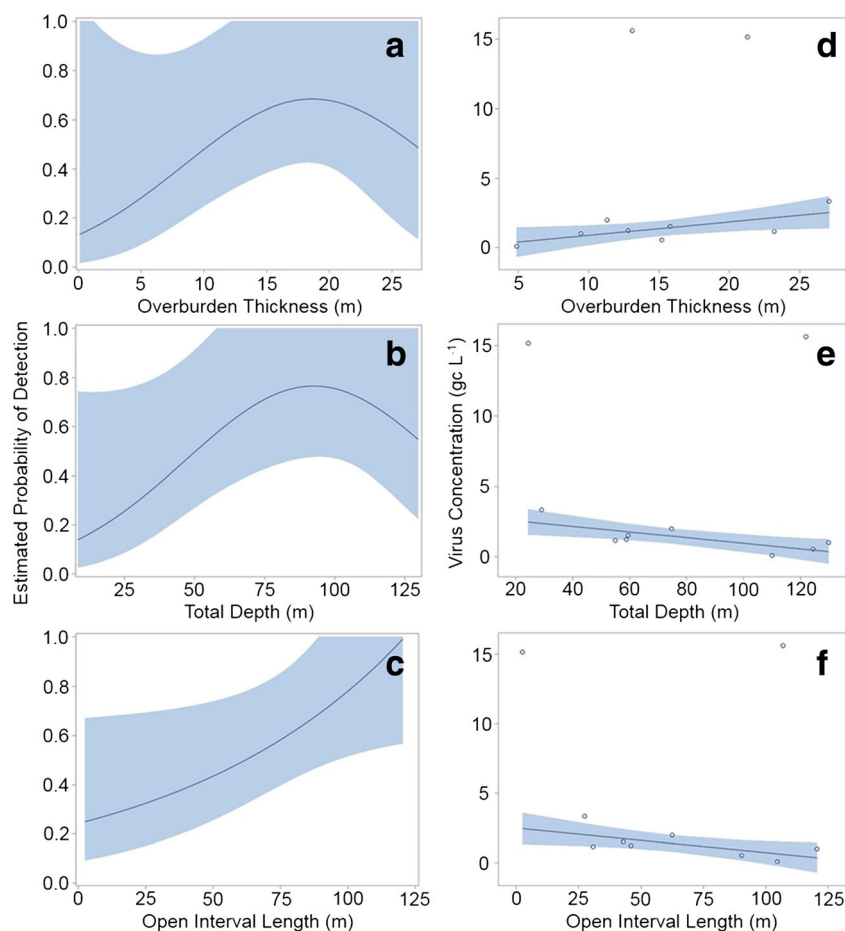
Overburden thickness was associated with virus concentration although, contrary to expectations, the association was positive. It was expected that the greater attenuating properties of thicker unsaturated overburden sediments would result in decreased virus concentrations in the underlying fractured bedrock aquifer. The positive correlation could be due to an unknown confounder or spurious from the small number of positive samples. In addition, overburden thickness at the well head (as was determined in this study) may not truly reflect well vulnerability as fractured bedrock aquifers are characterized by large capture zones that could have a contaminant source a substantial distance from the well. Assessing the importance of overburden thickness within entire capture zones and not just at the well head could lead to a better understanding of well vulnerability to virus contamination in fractured bedrock aquifers.

Open interval length of the wells was positively associated with the probability of detecting viruses. Wells with longer open intervals are more likely to capture more groundwater flow paths, increasing the likelihood that one flow path will include a virus source upgradient.

Open interval length and well depth were negatively associated with virus concentration. As most of the wells were only cased through the overburden and left open from the top of rock to the base of the well, deeper wells had longer open intervals. Well depth was indeed highly correlated with open interval length (Pearson and Spearman correlation coefficients = 0.93, $p < 0.0001$, $n = 22$). Deeper wells with longer open intervals have greater capacity for extracting groundwater (well yields) and dilution that could lower the concentration of any viruses present in the well. Furthermore, deeper wells will be extracting groundwater traveling along deeper and longer flow paths with longer residence times allowing attenuation of pathogens prior to reaching the well. The complexity of the three-dimensional (3-D) groundwater flow system with groundwater pumping from long open-well screens in fractured bedrock aquifers provides strong mixing of variable aged groundwater and, therefore, travel paths, leading to very low concentrations when detections occur. Hence, what is most striking for this study is the frequency of positive detects in a large number of wells but consistently at very low concentrations.

In this investigation, greater amounts of precipitation 8–14 days or 15–21 days previous to the sampling date were associated with higher virus concentrations in the virus positive wells. Precipitation can enhance virus transport by infiltrating water-carrying viruses downward to groundwater. Moreover, low ionic strength water, as is characteristic of precipitation, can desorb viruses that had been attached to sediment grains (Hunt and Johnson 2016). Bradbury et al. (2013) also demonstrated a strong association between precipitation events and increased virus detection rates, and heavy precipitation rates have

Fig. 5 Association of three well construction characteristics (overburden thickness, well depth, and open interval length) with the probability of virus detection (*left-panel plots: a–c*) and virus concentrations (*right-panel plots: d–f*). The evaluation of virus concentrations was restricted to the subset of wells that were virus-positive. *Blue-shading* represents the 95% confidence limits



been associated with increased incidence of acute gastrointestinal illness (Uejio et al. 2014).

While most of the statistical associations identified in the present study have reasonable mechanistic explanations or have been previously reported in the literature their interpretation still requires caution. The datasets used in the statistical analyses were small (10 virus positive samples in 22 wells, 7–36 sampling dates with ≥ 1 virus-positive well), which limited our ability to detect significant effects that may exist. The small sample size also limited our ability to account for the nonindependence of the data through methods such as mixed models or robust variance estimation. As a result, it is possible that some of the reported *p*-values are artificially low. The statistical modeling also did not include potentially important covariates, for example, proximity of the virus contamination source.

Virus transport to the study wells

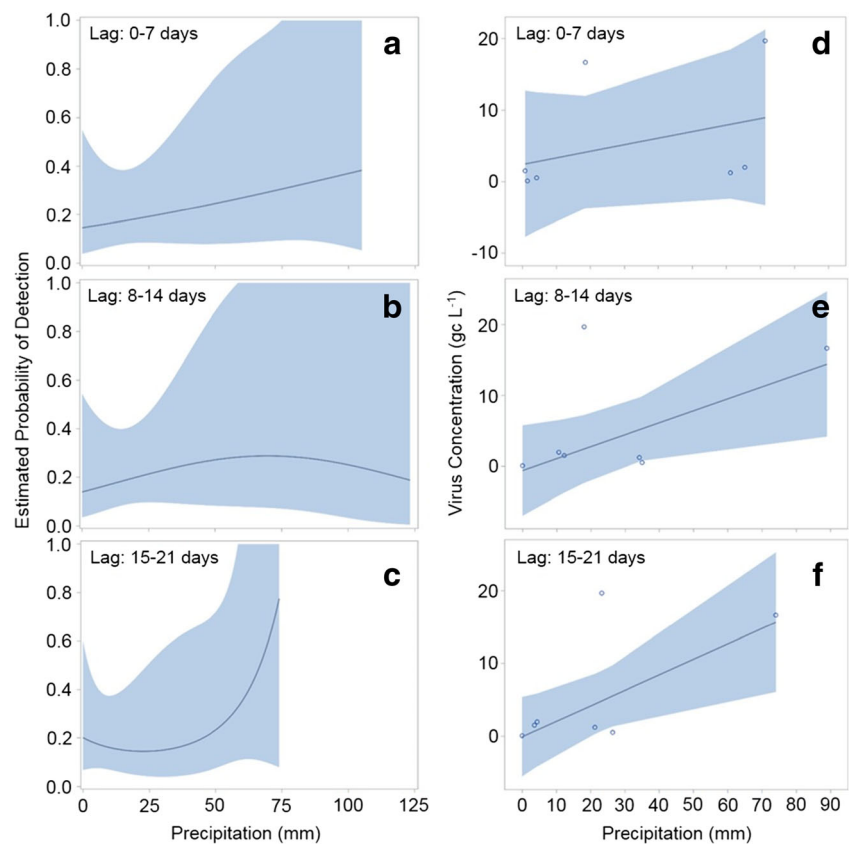
Determining capture zones and time of travel were outside the scope of the current study. On the other hand, both Ogorzaly et al. (2010) and Charles et al. (2009) showed the adenovirus genome (three positive wells) degraded within less than

2 years in groundwater and Bofill-Mas et al. (2006) showed human polyomavirus (four positive samples) to have persistence characteristics similar to adenovirus. This suggests that the bedrock wells in the southern Wellington County area that were positive for viruses were influenced by water less than 2 years old and were susceptible to rapid sewage-derived contamination.

Risk associated with virus contamination of a fractured bedrock aquifer

While numerous wells within the Guelph area exhibited positive virus detections throughout this study, the population is not necessarily at risk for waterborne disease transmission. In Ontario municipal water supplies must abide by the Safe Drinking Water Act and meet the Ontario Drinking Water Quality Standards. As these standards call for the treatment and chlorination of water distributed to the public as drinking water, viruses present in the supply wells are likely inactivated prior to distribution. Private well owners, however, are not required to treat or disinfect their drinking water. Although the infectious nature of the viruses detected in the current investigation

Fig. 6 Association of cumulative precipitation during the periods 0–7 days; 8–14 days; and 15–21 days prior to the sample collection date with the probability of virus detection (left-panel plots: **a–c**) and virus concentrations (right-panel plots: **d–f**). The evaluation of virus concentrations was restricted to the subset of sampling dates with ≥ 1 virus-positive well. Blue-shading represents the 95% confidence limits



was not determined, there may still be some risk associated with their detection. Borchardt et al. (2012) showed qPCR-measured enteroviruses and genogroup I noroviruses were strongly associated with acute gastrointestinal illness in communities supplied with non-disinfected groundwater.

Consumption of inadequately treated or raw groundwater in the study area may similarly result in elevated disease risk. As many private well owners drink untreated or minimally treated water, they may be at a risk of acute gastrointestinal illnesses derived from their drinking water.

Table 6 Private well construction and virus sample summary statistics from the current study and Borchardt et al. 2003

Study	Statistic	Well depth (m)	Casing depth (m)	Well age (year)	Sample volume (L)	Sample pH	Sample temp (°C)	Detection rate per sample	Detection rate per well
Allen 2013	<i>n</i>	11	11	11	63	66	64	5/63 (8%)	5/11 (45%)
	Mean	39.0	16.7	20.1	917	7.31	11.15		
	Median	31.1	15.8	22	897	7.28	10.79		
	Minimum	15.8	7.62	6	623	6.92	8.93		
	Maximum	74.7	27.4	36	1,377	7.82	18.85		
Borchardt et al. 2003	<i>n</i>	44 ^a	43	42	194	193	192	5/194 (3%)	4/50 ^b (8%)
	Mean	36.6	25.3	7	1,234	7.36	11.7		
	Median	33.2	17.1	6	1,325	7.41	11.6		
	Minimum	8.8	7.9	2	568	6.5	7.7		
	Maximum	93.0	73.2	22	1,605	8.54	18.6		

^a Of the 50 wells sampled in the Borchardt study, only 44 had well completion data

^b Of the 50 wells sampled in the Borchardt study, 30 were completed in fractured bedrock. The detection rate per well completed in fractured bedrock was 3/30, or 10%

Traditional fecal indicators and virus detection

In the United States, the Ground Water Rule (US EPA 2006) relies heavily on the requirements of the Total Coliform Rule (1989), which ultimately calls for the monitoring of the distribution systems of all public water systems for total coliforms at a frequency proportional to the number of people served by that water system. In Canada, the Safe Drinking Water Act requires similar monitoring of entry points to drinking water distribution systems (Government of Ontario, MOE, 2002); however, the regional Public Health authority, Public Health Wellington-Dufferin-Guelph (2016) recommends that private well owners sample their water 3 times per year. In the current study, virus samples were collected up to 6 times from some of the sampling wells while samples for bacterial indicators were only collected twice over 8 months; therefore, the results for the fecal indicators that are presented here are more representative of sampling frequencies of private wells than public water systems.

Of the 10 virus occurrences detected in the current study, 4 occurred during sampling events when bacterial samples were also being collected. Of these four occurrences, only one of the virus detections was accompanied by a bacterial indicator; the detection of Adenovirus A in MSW4 was accompanied by the detection of total coliforms at an average concentration of $0.043 \text{ CFU } 100 \text{ m L}^{-1}$. The other three virus detections went unaccompanied by traditional fecal indicators. Of the six virus detections that occurred without simultaneous sampling for fecal indicators, four of the wells that exhibited viruses had positive detections of total coliforms 1–4 months prior to the virus detection and only one of these detections was also positive for *E. coli*. Therefore, five of the 10 wells that exhibited virus detections also had detections of total coliforms at some point in time but the remainder of the virus detections went completely undetected by traditional fecal indicators. From these results, it is clear that analyzing water for total coliforms and *E. coli* at the frequency suggested by Public Health Ontario can miss up to 50% of fecal contamination incidents.

The fact, however, that total coliforms and *E. coli* are not 100% efficient indicators of virus contamination is not a new concept (Keswick and Gerba 1980; Lindsey et al. 2002; Borchardt et al. 2003). As these bacteria can be up to 100 times larger than viruses, their transport properties in fractured bedrock aquifers can be significantly different. What makes the statistics presented here interesting is the fact that the sample volumes collected during this investigation were 10 times greater than typical sampling methods would suggest; as the concentrations of the fecal indicators were expected to be low, sample volumes were increased to approximately 1,000 ml in comparison to the 100 ml suggested by Method 1604 (US EPA 2002). This proved to be a valuable modification as the maximum concentration of the triplicate samples collected at MSW4 was $0.147 \text{ CFU } 100 \text{ m L}^{-1}$ (1 colony 680 m L^{-1}),

which is likely to have been missed if only the suggested 100-ml sample had been collected. In addition, total coliforms were detected in a total of nine wells during both bacterial sampling events. Of the nine detections during the first event, seven detections had average concentrations less than $1 \text{ CFU } 100 \text{ m L}^{-1}$. The second sampling event exhibited 6 detections with average concentrations less than $1 \text{ CFU } 100 \text{ m L}^{-1}$. For *E. coli*, three out of the four detections for both sampling events had average concentrations less than $1 \text{ CFU } 100 \text{ m L}^{-1}$. This would suggest that even with high frequency sampling of large public water systems, fecal contamination could go undetected if 100-ml sample volumes are continued to be used.

Conclusions

During an 8-month sampling campaign with six sampling events per well, human enteric viruses were found in five of eight municipal supply wells and five of 11 private wells in the fractured dolostone aquifer of Southern Wellington County. Wells with longer open interval lengths were more likely to be virus positive. In the virus-positive wells, virus concentrations decreased with increasing well depths and longer open interval lengths. Virus concentrations increased with increasing amounts of precipitation in the periods 8–14 days or 15–21 days prior to the sample date. Fractured bedrock aquifers are known for their relatively high degree of vulnerability to contamination due to rapid groundwater flow rates in well-connected fracture networks, and as previous investigations reported in the literature would suggest, also vulnerable to virus contamination. As far as the authors are aware, this study reports the most comprehensive sampling for enteric viruses in a fractured dolostone aquifer. Such aquifers in regions like southern Wellington County where the overburden is thin in groundwater recharge areas, well casings are shallow and extend only into the upper few feet of bedrock, the regional water table is shallow, groundwater velocities in the fractures are $1\text{--}10 \text{ m day}^{-1}$, and cool groundwater temperatures promote virus longevity are all characteristics in combination that create an ideal hydrogeologic setting for virus transport and widespread contamination, although at very low concentrations.

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